

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization International Bureau

ZIPO OMPI

- Programme de la company de la company

(43) International Publication Date 22 January 2004 (22.01.2004)

PCT

(10) International Publication Number WO 2004/007661 A3

(51) International Patent Classification⁷: 1/04, 1/06

C07K 1/02,

(21) International Application Number:

PCT/IB2003/005473

(22) International Filing Date: 9 J

9 June 2003 (09.06.2003)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data: 60/387,825

10 June 2002 (10.06.2002) US

(71) Applicant (for all designated States except US): GENE-PROT, INC. [CH/CH]; 2, Pre-de-la-Fontaine, CP 125, Meyrin 2, CH-1217 Geneva (CH).

(72) Inventors; and

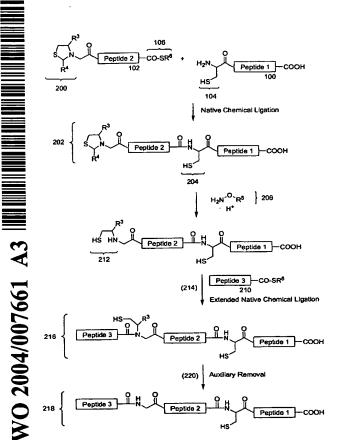
(75) Inventors/Applicants (for US only): BOTTI, Paolo

[IT/CH]; * (CH). VILLAIN, Matteo [IT/CH]; * (CH). MANGANIELLO, Sonia [IT/FR]; * (FR). GAERTNER, Hubert [FR/FR]; * (FR).

- (74) Agent: THOMSON, Paul, A.; Potts, Kerr & Co., 15 Hamilton Square, Birkenhead, Merseyside L41 6BR (GB).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,

[Continued on next page]

(54) Title: CARBOXY PROTECTION STRATEGIES FOR ACIDIC C-TERMINAL AMINO ACIDS IN CHEMICAL LIGATION OF OLIGOPEPTIDES



(57) Abstract: The present invention provides methods of assembling oligopeptides or polypeptides in a native chemical ligation reaction that eliminates the formation of unwanted side products resulting from the presence of an unprotecte acidic C-terminal oligopeptide thioester. An important aspect of the present invention is providing side chain protected acidic C-terminal oligopeptide thioesters. The present invention is useful in methods for chemical synthesis of oligopeptides, polypeptides and proteins and improves the efficiency of native chemical ligation reactions, particularly where aspartyl or glutamyl peptide fragments are used to assemble an oligopeptide, polypeptide or protein product.







SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv)) for US only

Published:

with international search report

(88) Date of publication of the international search report: 22 July 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

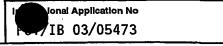


In	nal	Application No
PCT/	ΙB	03/05473

A. CLASSIF IPC 7	FICATION OF SUBJECT MATTER C07K1/02 C07K1/04 C07K1/	' 06	
According to	International Patent Classification (IPC) or to both national class	sification and IPC	
B. FIELDS			
	cumentation searched (classification system followed by classifi CO7K	ication symbols)	
	ion searched other than minimum documentation to the extent th		
	ata base consulted during the international search (name of data BS Data, BIOSIS	a base and, where practical, search terms used)
C. DOCUME	ENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the	e relevant passages	Relevant to claim No.
A	T M HACKENG ET AL.: "Protein shative chemical ligation: expandy using straightforward method PROCEEDINGS OF THE NATIONAL ACASCIENCES OF USA., vol. 96, 1999, pages 10068-100 XP002940700 NATIONAL ACADEMY OF SCIENCE. W. US ISSN: 0027-8424 the whole document	nded scope dology " ADEMY OF 73,	1-11
X Furt	ther documents are listed in the continuation of box C.	Patent family members are listed	in annex.
"A" docum consister filing "L" docum which citatic "O" docum other "P" docum later t	ategories of cited documents: nent defining the general state of the art which is not dered to be of particular relevance document but published on or after the international date eent which may throw doubts on priority claim(s) or is cited to establish the publication date of another on or other special reason (as specified) ment referring to an oral disclosure, use, exhibition or means eent published prior to the international filing date but than the priority date claimed	"T" later document published after the interpretation or priority date and not in conflict with cited to understand the principle or the invention. "X" document of particular relevance; the cannot be considered novel or cannot involve an inventive step when the discurrent of particular relevance; the cannot be considered to involve an indocument is combined with one or ments, such combination being obvious the art. "&" document member of the same patern.	h the application but heory underlying the claimed invention of the considered to locument is taken alone claimed invention nventive step when the nore other such docu-ous to a person skilled
2	25 March 2004	16/04/2004	
Name and	mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Masturzo, P	

Category* Citation of document, with indication, where appropriate, of the relevant passages A Y S LEE ET AL.: "A convergent 11quid-phase synthesis of salmon calcitonin" JOURNAL OF PEPTIDE RESEARCH., vol. 54, 1999, pages 328-335, XP002274977 MUNKSGAARD, COPENHAGEN., DK ISSN: 0367-8377 the whole document A R B WOODWARD ET AL.: "The total synthesis of cyclosporin C" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol. 88, no. 4, 20 February 1966 (1966-02-20), pages 852-853, XP002274978 AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US ISSN: 0002-7863 the whole document A F AL-OBEIDI ET AL.: "Synthesis of beta-and gamma-fluorenylmethyl esters of respectively N-alpha-Boc-L-aspartic acid and N-alpha-Boc-L-glutamic acid" INTERNATIONAL JOURNAL OF PEPTIDE AND PROTEIN RESEARCH., vol. 35, 1990, pages 215-218, XP002274979 MUNKSGAARD, COPENHAGEN., DK ISSN: 0367-8377 the whole document A G C STELAKATOS ET AL.: "New methods in peptide synthesis. Part III. Protection of carboxyl group" JOURNAL OF THE CHEMICAL SOCIETY, SECTION C: ORGANIC CHEMISTRY, no. 13, 1966, pages 1191-1199, XP002274980 CHEMICAL SOCIETY. LETCHWORTH., GB ISSN: 0022-4952 the whole document A B CLIPPINGDALE ET AL.: "Peptide 1-1	
A Y S LEE ET AL.: "A convergent liquid-phase synthesis of salmon calcitonin" JOURNAL OF PEPTIDE RESEARCH., vol. 54, 1999, pages 328-335, XP002274977 MUNKSGARD, COPENHAGEN., DK ISSN: 0367-8377 the whole document A R B WOODWARD ET AL.: "The total synthesis of cyclosporin C" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol. 88, no. 4, 20 February 1966 (1966-02-20), pages 852-853, XP002274978 AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US ISSN: 0002-7863 the whole document A F AL-OBEIDI ET AL.: "Synthesis of beta-and gamma-fluorenylmethyl esters of respectively N-alpha-Boc-L-aspartic acid and N-alpha-Boc-L-glutamic acid" INTERNATIONAL JOURNAL OF PEPTIDE AND PROTEIN RESEARCH., vol. 35, 1990, pages 215-218, XP002274979 MUNKSGARD, COPENHAGEN., DK ISSN: 0367-8377 the whole document A G C STELAKATOS ET AL.: "New methods in peptide synthesis. Part III. Protection of carboxyl group "JOURNAL OF THE CHEMICAL SOCIETY, SECTION C: ORGANIC CHEMISTRY., no. 13, 1966, pages 1191-1199, XP002274980 CHEMICAL SOCIETY. LETCHWORTH., GB ISSN: 0022-4952 the whole document A B CLIPPINGDALE ET AL.: "Peptide 1-1	
liquid-phase synthesis of salmon calcitonin" JOURNAL OF PEPTIDE RESEARCH., vol. 54, 1999, pages 328-335, XP002274977 MUNKSGAARD, COPENHAGEN., DK ISSN: 0367-8377 the whole document A R B WOODWARD ET AL.: "The total synthesis of cyclosporin C" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol. 88, no. 4, 20 February 1966 (1966-02-20), pages 852-853, XP002274978 AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US ISSN: 0002-7863 the whole document A F AL-OBEIDI ET AL.: "Synthesis of beta- and gamma-fluorenylmethyl esters of respectively N-alpha-Boc-L-aspartic acid and N-alpha-Boc-L-glutamic acid" INTERNATIONAL JOURNAL OF PEPTIDE AND PROTEIN RESEARCH., vol. 35, 1990, pages 215-218, XP002274979 MUNKSGAARD, COPENHAGEN., DK ISSN: 0367-8377 the whole document A G C STELAKATOS ET AL.: "New methods in peptide synthesis. Part III. Protection of carboxyl group " JOURNAL OF THE CHEMICAL SOCIETY, SECTION C: ORGANIC CHEMISTRY., no. 13, 1966, pages 1191-1199, XP002274980 CHEMICAL SOCIETY LETCHWORTH., GB ISSN: 0022-4952 the whole document A B CLIPPINGDALE ET AL.: "Peptide	
of cyclosporin C" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol. 88, no. 4, 20 February 1966 (1966-02-20), pages 852-853, XPO02274978 AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US ISSN: 0002-7863 the whole document A F AL-OBEIDI ET AL.: "Synthesis of beta- and gamma-fluorenylmethyl esters of respectively N-alpha-Boc-L-aspartic acid and N-alpha-Boc-L-glutamic acid" INTERNATIONAL JOURNAL OF PEPTIDE AND PROTEIN RESEARCH., vol. 35, 1990, pages 215-218, XPO02274979 MUNKSGAARD, COPENHAGEN., DK ISSN: 0367-8377 the whole document A G C STELAKATOS ET AL.: "New methods in peptide synthesis. Part III. Protection of carboxyl group " JOURNAL OF THE CHEMICAL SOCIETY, SECTION C: ORGANIC CHEMISTRY., no. 13, 1966, pages 1191-1199, XPO02274980 CHEMICAL SOCIETY. LETCHWORTH., GB ISSN: 0022-4952 the whole document A A B CLIPPINGDALE ET AL.: "Peptide 1-1	
and gamma-fluorenylmethyl esters of respectively N-alpha-Boc-L-aspartic acid and N-alpha-Boc-L-glutamic acid" INTERNATIONAL JOURNAL OF PEPTIDE AND PROTEIN RESEARCH., vol. 35, 1990, pages 215-218, XP002274979 MUNKSGAARD, COPENHAGEN., DK ISSN: 0367-8377 the whole document A G C STELAKATOS ET AL.: "New methods in peptide synthesis. Part III. Protection of carboxyl group " JOURNAL OF THE CHEMICAL SOCIETY, SECTION C: ORGANIC CHEMISTRY., no. 13, 1966, pages 1191-1199, XP002274980 CHEMICAL SOCIETY. LETCHWORTH., GB ISSN: 0022-4952 the whole document A B CLIPPINGDALE ET AL.: "Peptide 1-1	
peptide synthesis. Part III. Protection of carboxyl group " JOURNAL OF THE CHEMICAL SOCIETY, SECTION C: ORGANIC CHEMISTRY., no. 13, 1966, pages 1191-1199, XP002274980 CHEMICAL SOCIETY. LETCHWORTH., GB ISSN: 0022-4952 the whole document A B CLIPPINGDALE ET AL.: "Peptide 1-1	
1/1 1 1/ 0 0 0 0 1/4 0 1/	1
thioester preparation by FMOC solid phase peptide synthesis for use in native chemical ligation" JOURNAL OF PEPTIDE SCIENCE., vol. 6, no. 5, pages 225-234, XP009015362 JOHN WILEY AND SONS LTD., GB ISSN: 1075-2617 the whole document	1





Citeopry* Citation of document, with indication, where appropriate, of the relevant passages Citation of document, with indication, where appropriate, of the relevant passages
T M VILLAIN ET AL.: "Native chemical ligation with aspartic ang glutamic acids as C-terminal residues: scope and limitations." EUROPEAN JOURNAL OF ORGANIC CHEMISTRY., no. 17, 2003, pages 3267-3272, XP002274981 WILEY-VCH, WEINHEIM., DE ISSN: 1434-193X the whole document P,X M VILLAIN ET AL.: "Backbone isomerization at aspartic-, glutamic acid-cysteine ligation " JOURNAL OF PEPTIDE SCIENCE., vol. 8 (suppl.), 31 August 2002 (2002-08-31), page s108 XP009015060 JOHN WILEY AND SONS LTD., GB ISSN: 1075-2617
ligation with aspartic ang glutamic acids as C-terminal residues: scope and limitations." EUROPEAN JOURNAL OF ORGANIC CHEMISTRY., no. 17, 2003, pages 3267-3272, XP002274981 WILEY-VCH, WEINHEIM., DE ISSN: 1434-193X the whole document P,X M VILLAIN ET AL.: "Backbone isomerization at aspartic-, glutamic acid-cysteine ligation " JOURNAL OF PEPTIDE SCIENCE., vol. 8 (suppl.), 31 August 2002 (2002-08-31), page s108 XP009015060 JOHN WILEY AND SONS LTD., GB ISSN: 1075-2617
at aspartic-, glutamic acid-cysteine ligation " JOURNAL OF PEPTIDE SCIENCE., vol. 8 (suppl.), 31 August 2002 (2002-08-31), page s108 XP009015060 JOHN WILEY AND SONS LTD., GB ISSN: 1075-2617